The Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study (PROUDEST): A Multicenter Prospective Cohort Study Protocol

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Abstract

Background: A growing body of evidence suggests that infection by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) during pregnancy may affect maternal-fetal outcomes, with possible implications for the long-term development of exposed children.

Objective: The PRegnancy OUtcomes and child Development Effects of SARS-CoV-2 infection STudy (PROUDEST) is a multicenter prospective study designed to understand the repercussions of COVID-19 to mother-child global health.

Methods: The PROUDEST study comprises two prospective sequential substudies. The PREGNANT substudy will assess the effects of SARS-CoV-2 infection on pregnancy, childbirth and puerperium clinically and from a mechanistic standpoint to understand the inflammatory and immunological phenomena underlying COVID-19 in relation to pregnancy. Pregnant women aged 18 to 40 years with laboratory-proven exposure to SARS-CoV-2 (group A, n = 300) will be compared to control subjects with no laboratory evidence of in-pregnancy exposure to the virus (group B, n = 300). Subjects exposed to other infections during pregnancy will be excluded. The BORN substudy is a long-term follow-up study assessing the offspring of women who entered the prior substudy. It will describe the effects of SARS-CoV-2 exposure during pregnancy on children's growth, neurodevelopment and metabolism from birth up to five years of age. It includes two comparison groups: group A (exposed, n = 300) comprises children born from SARS-CoV-2-exposed pregnancies, and group B (controls, n = 300) comprises children from nonexposed mothers.

Results: Recruitment began in July 2020, and as of September 2020, 115 pregnant women infected with SARS-CoV-2 during pregnancy and 80 newborns had been included. Data
analysis is scheduled to start after all data have been collected.

Conclusions: Upon completion of the study, we expect to have obtained comprehensive data to provide a better understanding of

the effects of SARS-CoV-2 and its inflammatory and immunological processes on pregnancy, puerperium and infancy. Our findings will inform clinical decisions regarding the care of exposed mothers and children and support the development of evidence-based public health policies. Clinical Trial: The PROUDEST study was retrospectively registered on the Brazilian Register of Clinical Trials website (REBEC, https://ensaiosclinicos.gov.br/rg/RBR-65qxs2/, study ID RBR-65qxs2.

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Abstract

Background: A growing body of evidence suggests that infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) during pregnancy may affect maternal-fetal outcomes, with possible implications for the long-term development of exposed children.

Objective: The PRegnancy OUtcomes and child Development Effects of SARS-CoV-2 infection STudy (PROUDEST) is a multicenter prospective study designed to elucidate the repercussions of COVID-19 for mother-child global health.

Methods: The **PROUDEST** trial comprises two prospective sequential substudies. The PREGNANT substudy will assess the effects of SARS-CoV-2 infection on pregnancy, childbirth and puerperium clinically and from a mechanistic standpoint to elucidate the inflammatory and immunological phenomena underlying COVID-19 in relation to pregnancy. Pregnant women aged 18 to 40 years with laboratory-proven exposure to SARS-CoV-2 (group A, n = 300) will be compared to control subjects with no laboratory evidence of in-pregnancy exposure to the virus (group B, n = 300). Subjects exposed to other infections during pregnancy will be excluded. The BORN substudy is a long-term follow-up study assessing the offspring of women who entered the prior substudy. It will describe the effects of SARS-CoV-2 exposure during pregnancy on children's growth, neurodevelopment and metabolism from birth up to five years of age. It includes two comparison

groups: group A (exposed, n = 300) comprises children born from SARS-CoV-2-exposed pregnancies, and group B (controls, n = 300) comprises children from nonexposed mothers.

Results: Recruitment began in July 2020, and as of September 2020, 115 pregnant women infected with SARS-CoV-2 during pregnancy and 80 newborns had been included. Data analysis is scheduled to start after all data have been collected.

Conclusions: Upon completion of the study, we expect to have obtained comprehensive data to provide a better understanding of the effects of SARS-CoV-2 and its inflammatory and immunological processes on pregnancy, puerperium and infancy. Our findings will inform clinical decisions regarding the care of exposed mothers and children and support the development of evidence-based public health policies.

Trial Registration: The PROUDEST study was registered on the Brazilian Register of Clinical Trials website (https://www. http://www.ensaiosclinicos.gov.br), ID RBR65QXS2, on June 13, 2020, where Brazilian clinical trials are exclusively registered.

Keywords: SARS-CoV-2; COVID-19; pregnancy; neonate; children

Background

The natural history of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is still being written. SARS-CoV-2-infected individuals may present with a broad spectrum of clinical manifestations, from no symptoms to dramatically progressive disease, eventually leading to death [1, 2]. Some individuals develop an intense inflammatory and procoagulant process that can result in severe pulmonary damage, the main cause of COVID-19 morbidity and mortality.

The pathophysiological phenomena taking place in other potentially SARS-CoV-2-targeted human tissues need further investigation. A central nervous system (CNS) viral tropism has been

postulated based on reports of neurological events such as stroke, acute hemorrhagic encephalopathy, seizures and loss of smell and taste [3, 4].

To date, little is known about the effects of COVID-19 on women during pregnancy and puerperium [5, 6] or its consequences in their offspring, from the neonatal period throughout the first years of life [7-9]. Thus, a robust evidence base for the proper management of these mothers and children is still lacking.

Experience with other previous epidemics of viral-induced respiratory distress syndrome have shown that pregnant and puerperal women have higher risks of developing life-threatening clinical presentations [10]. This generally worse outcome has been attributed to physiological changes in the immune and cardiopulmonary systems during pregnancy [11]. Examples of epidemics include the H1N1 influenza, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) epidemics, in which pregnancy mortality rates reached 27% [10, 12, 13].

Nevertheless, despite the structural similarity between the coronaviruses, the initial reports of COVID-19 in pregnant women showed lower rates of intensive care unit (ICU) admission, orotracheal intubation and death for the SARS-CoV-2 outbreak than for the SARS-CoV and MERS-CoV outbreaks [9, 13]; however, more recent papers have shown higher morbidity and mortality rates among pregnant women than among nonpregnant women. Several aspects of embryo implantation [14], placental development [15] and delivery dynamics [16] seem to be impaired by the inflammatory response driven by immune cell subtypes at the maternal-fetal interface [17]. Such phenomena may precipitate preeclampsia, spontaneous abortion, intrauterine growth restriction, and premature birth [18-21].

Most available data were obtained from women exposed to SARS-CoV-2 during the second half of pregnancy. Thus, SARS-CoV-2 infection during all stages of pregnancy, including the early stages of gestation, has not been fully investigated. Nevertheless, as the disease spreads globally,

more women are being exposed to the virus during early and mid-gestation, and new data have been accruing [22].

Studies evaluating the vertical transmission of SARS-CoV-2 are still inconclusive [6, 23-25]. Investigations of placentas from SARS-CoV-2-infected women have suggested the low likelihood of viral transplacental transmission, although potentially hazardous effects of the inflammatory and prothrombotic environment on placental function and, consequently, fetal growth could not be ruled out [26-28].

To date, the few reports on postnatally infected neonates have shown a trend towards asymptomatic involvement or mild clinical forms with favorable outcomes, although the younger the infant is, the higher the risk of a more critical picture [22, 29].

Maternal SARS-CoV-2 infection would potentially expose the fetus not only to direct viral effects but also to the placental inflammatory response and to the maternal cytokine storm [5, 7, 22]. Such processes and their consequences have not been extensively studied. Understanding these phenomena should contribute to the proper management of children born to SARS-CoV-2-infected mothers.

A case series on the clinical aspects of newborns of COVID-19-exposed mothers reported a low risk of adverse outcomes for late pregnancy exposure while stating the paramount need for close follow-up [30]. Other reports have presented data showing no adverse effects on neonates born to mothers who tested positive for COVID-19. Liu et al. [31] described nineteen completely asymptomatic neonates in Wuhan (China).

On the other hand, a few studies reported that SARS-CoV-2 test-negative neonates born to mothers who tested positive and developed critical illness might present ominous clinical profiles, suggesting the potential impact of the inflammatory processes on fetal physiology. Romagano et al. [32] reported a prevalence rate of 6.9% for symptomatic SARS-CoV-2-positive pregnant women among 1,053 deliveries in a New Jersey (USA) large hospital network. Eight pregnant women were

critically ill, and seven neonates tested negative (RT-PCR), with one not yet delivered at the time of testing. All were preterm and appropriate for gestational age, except one (small for gestational age). They were all separated from their mothers after delivery, and all of them developed respiratory distress and required neonatal ICU. Anemia and hyperbilirubinemia of prematurity, temperature instability and feeding problems were reported in some of those neonates.

Several other papers reported symptoms among test-negative neonates born to COVID-19-positive mothers, such as rashes, facial ulceration and the need for noninvasive oxygen support [33]; transient lymphocytopenia and impaired liver function [34]; disseminated intravascular coagulation; and even multiple organ failure leading to death [35]. There are many critical questions yet to be answered regarding the standards of care for SARS-CoV-2-exposed pregnant women and their offspring, and guidelines are still being developed around the world [8, 36, 37]. Therefore, the overall purpose of this study is to describe the effects of in-pregnancy SARS-CoV-2 infection and its related inflammatory and immunological phenomena on the health of exposed women and their offspring.

Objective

The specific aims are (a) to study the effects of COVID-19 on maternal and obstetric morbidity and mortality, including indicators such as preeclampsia, abortion, fetal malformation, fetal growth, and premature birth; (b) to investigate the presence of SARS-CoV-2 and/or anti-SARS-CoV-2 antibodies in the cerebrospinal fluid (CSF) of women with symptomatic COVID-19 undergoing spinal anesthesia for cesarean section; (c) to determine the profiles of serum proinflammatory and regulatory cytokines in pregnant women with symptomatic COVID-19; (d) to determine the profiles of CSF proinflammatory and regulatory cytokines of symptomatic COVID-19-positive women undergoing spinal anesthesia for cesarean section; (e) to study histopathological markers of inflammatory and thrombotic phenomena in the placenta; (f) to study the correlation between the aforementioned serum and histologic biomarkers of COVID-19 with the outcomes of

pregnancy, delivery, puerperium and childbirth, as well as with short- and long-term health outcomes during infancy; (g) to study the association between use of maternal pharmacological therapy directed at COVID-19 and their offspring's health outcomes; (h) to evaluate the effects of COVID-19 exposure, according to stage of pregnancy in which it occurred, on fetal, neonatal and infantile morbidity and mortality; and (i) to evaluate the effects of in-pregnancy COVID-19 exposure on children's somatic and neurological development and energy metabolism up to five years of age.

Methods

The **PR**egnancy **OU**tcomes and child **D**evelopment **E**ffects of **S**ARS-CoV-2 infection **ST**udy (PROUDEST) is a multicenter, longitudinal, prospective, observational study conducted in two sequential stages, the PREGNANT and BORN branches or substudies, each with two parallel groups (exposed and nonexposed) for comparisons. The PROUDEST study is designed to address the multifaceted question about the impact of COVID-19 exposure during pregnancy on the global health of the mother-child dyad (Figure 1).

The PREGNANT substudy will follow up – until day 21 postpartum – pregnant women exposed to SARS-CoV-2 at any phase of gestation, compared to a control group of nonexposed pregnant women. The BORN substudy will follow up the children of the women included in the preceding (PREGNANT) branch. These children will also be allocated into two comparison groups (exposed and nonexposed) according to their mothers' in-pregnancy exposure status and will be followed up by a multidisciplinary team of health professionals from birth up to the age of 5 years through regular consultations every month up to 6 months old, every 3 months up to 2 years old and every 6 months up to 5 years old. Mothers and children may attend nonscheduled visits as needed for clinical reasons, as well as specific appointments for conducting the procedures and tests described in this protocol.

The PROUDEST study will be conducted from July 2020 to December 2026 in Brasília,

Brazil. The recruitment of pregnant and newborn dyads will be carried out using data from the Epidemiological Surveillance Center of the Federal District. Follow-up of these mother-fetus dyads will be carried out up to childbirth (and puerperium, in the case of mothers) until December 2021, when the last included dyads are expected to reach delivery in two different hospitals: University Hospital of Brasília and Asa Norte Regional Hospital (the reference public medical center for COVID-19 in the Federal District, Brazil), both placed in central urban areas, included in Brazilian public health system (SUS) and that primarily serve the low-income population. Thus, the results of the PREGNANT substudy will be published as soon as the analyses are completed. The children will be followed up from childbirth up to December 2026, when the last admitted neonate will turn five years old. As the BORN substudy is lengthy, partial results may be disclosed during the course of the study, but the final data will be available only in the second half of 2026.

Pregnant women entering the study must be over 18 years of age. COVID-19 exposure will be defined as a first-time RT-PCR, serology and/or rapid test that is positive during pregnancy, with confirmation by a second test. Nonexposure to COVID-19 will be defined as asymptomatic pregnant women with serology test (IgG,IgM) negative, which will be done ate 14-21 days post-partum.

Pregnant women with pre-existing chronic diseases or continuous medications (except diabetes and hypertension), who consume tobacco, alcohol or other drugs, or who have other suspected or confirmed congenital infections will be excluded.

Neonates whose mothers would qualify for inclusion in the PREGNANT substudy (had these women been screened) may also be admitted to the BORN substudy, even if their mothers missed participation in the preceding branch.

Children initially assigned to the control (nonexposed) group who are later confirmed to have become infected with SARS-CoV-2 during follow-up will be excluded from all analyses from the time of that diagnosis onward, although they will continue to receive assistance under the same standards until the end of the study.

Sample Size Calculation

No precise data are available on the prevalence of SARS-CoV-2 infection among pregnant women in Brazil, but international reports estimate that up to 15.3% of all pregnancies have been exposed [38]. Recent data indicate a birth rate of 44,195 newborns per year in the Federal District [39]. Thus, considering an “infinite” population (>20,000 pregnant women) and assuming a 15% prevalence of SARS-CoV-2-exposed pregnancies, a confidence level of 95% and margin of error of 5%, the minimum size for a random sample of exposed women would be 195, yielding an expected similar figure for the exposed children. If we set the expected dropout rate for the BORN substudy to 20%, the required number of exposed mothers (to give birth to the BORN participants) would increase to 234.

Nevertheless, our sampling approach is not truly random but based on convenience, as eligible subjects present to the recruitment centers. The aforementioned calculations serve only as a reference to avoid overestimating the inclusion of participants. Given the limited available knowledge regarding the effects of SARS-CoV-2 on pregnancy and child development, thus causing the study to have an eminently exploratory character, we adopted an approach of “as much as feasible, but no more than reasonable” for the sample size definition.

All considered, we set an *a priori* number of 300 exposed women in the PREGNANT phase, which would result in the expected 300 exposed children in the BORN phase. We adopted a 1:1 allocation rate between exposed and control subjects, thus suggesting the need for an additional 300 mothers and 300 children to constitute the nonexposed control groups. Hence, the overall sample size of the PROUDEST study was finally set to 1200 participants (600 mother-child dyads: 300 exposed, 300 control dyads).

To promote participant retention and complete follow-up at Pregnancy and Pediatrics Outpatient Clinics, we will actively search for patients by phone and email.

Procedures

A host of clinical, psychological, neurodevelopmental, biochemical, histological and imaging assessments will be conducted within the PROUDEST protocol, as described below. (Figure 2)

Prenatal data from both the pregnant women and their fetuses will be gathered during follow-up in Pregnancy Outpatient Clinic at the University Hospital of Brasilia. They will consist of the medical and sociodemographic data of the mothers; gestational age; symptoms, interventions and outcomes related to COVID-19 (for the exposed ones); screening for congenital infections; hypertensive disorders and other pregnancy-specific morbidities; general health assessment; general physical examination; routine clinical biochemistry tests; and ultrasound scans performed between gestational ages of 11 to 13 weeks and 6 days, 22 to 24 weeks and monthly in the third trimester of pregnancy to assess fetal growth and morphology, placental morphology, amniotic fluid volume, and dopplerfluxometry. Maternal blood will be collected at the first prenatal consultation, regardless of gestational age. Antenatal consultations will occur monthly up to week 34; every 2 weeks, between weeks 34 and 36; and then weekly up to delivery. During pregnancy, screening for psychological risk assessment and existence of psychic suffering, through the Beck Depression Inventory (BDI) will be performed in the first prenatal consultation. [40]. Individual psychological care will be given to pregnant women who have a Score> 12 in the BDI inventory. Mothers will also be evaluated physically and psychologically between days 7 and 21 postpartum by PREGNANT substudy and after by BORN substudy..

At childbirth, assessments will be conducted regarding the occurrence of dysfunctional labor, premature rupture of membranes, type of birth, delivery outcomes, physical examination and classification of the newborn, anthropometry, early initiation of breastfeeding, need for neonatal intensive care and type of interventions. Maternal blood, cerebrospinal fluid (CSF) from women undergoing spinal anesthesia for cesarean section and umbilical cord blood samples will be collected.

Cerebrospinal fluid will be collected immediately before the infusion of the medicine for

spinal anesthesia, in a sterile syringe, at an average dosage of 0.5 ml, and transported to a 4-mL cryotube. Blood samples of the mother and the umbilical cord will be collected in a heparinized tube, centrifuged immediately and the plasma will be stored in 4-mL sealed cryotubes. Both will be subsequently stored at -80 degrees Celsius for later analysis.

Assessment of blood cell counts (BCCs), inflammation markers (C reactive protein – CRP - and procalcitonin), biochemistry (ALT, AST, ferritin, alkaline phosphatase, lactic dehydrogenase) and SARS-CoV-2 tests (RT-PCR, IgM and IgG antibodies) will be carried out.

Circulating cytokine levels will be evaluated with the Luminex Bio-Plex Pro™ human cytokines 27 platform (Bio-Rad Laboratories, California, USA). The cytokine profile assessment will comprise chemokines (CXCL8, CCL11, CCL3, CCL4, CCL2, CCL5, CXCL10), proinflammatory cytokines (IL-1β, IL-6, TNF-, IL-12p70, IFN-gamma, IL-17A, IL-15), regulatory cytokines (IL-1Ra, IL-4, IL-5, IL-9, IL-10, IL-13) and cell growth factors (IL-2, IL-7, FGF-basic, PDGF, VEG, G-CSF and GM-CSF). All procedures will be performed according to the manufacturer's recommendations.

Analysis will be performed at the clinical biochemistry laboratories from the hospitals where delivery occurred (BCC, CRP, procalcitonin, routine biochemistry), the Central Laboratory from the Federal District Department of Health - LACEN (SARS-CoV-2 tests), and at University of Brasilia laboratory (cytokine profiles).

The placenta will be submitted to fresh histopathological analyses to assess possible morphological and histological changes that may be associated with SARS-CoV2 infection, according to Amsterdam protocol. [41].

Peripheral blood and cerebrospinal fluid (CSF) samples from the newborn will be collected only if there is a clinical need, not per routine research protocol. Nevertheless, if such specimens are made available, they will also be subjected to the aforementioned analyses.

All newborns will undergo neonatal screening tests in accordance with the recommendations of the Ministry of Health of Brazil. Five drops of blood will be collected on filter paper for the

national neonatal screening program. The sample will be collected after 48 hours of life, and the diseases screened will be phenylketonuria, congenital hypothyroidism, biotinidase deficiency, cystic fibrosis and congenital adrenal hyperplasia. After 24 hours of life and before discharge, a pulse oximetry test will be performed. Oxygen saturation will be measured in the right upper limb and one of the lower limbs. The test will be considered normal if the oxygen saturation is greater than or equal to 95% and the difference between the limbs is not greater or equal to 3%. Hearing screening will be performed between 36 and 48 hours of life by testing otoacoustic emissions.

After hospital discharge, all neonates will be followed up in the Pediatric Outpatient Clinic at the University Hospital of Brasilia. Child growth and neurodevelopment will be assessed at all visits. The first visit will be scheduled to occur on day 15 postpartum; then, visits will be monthly during the first six months of life. Thereafter, visits will be scheduled every three months up to 12 months of age and every six months up to 5 years of age. Nonscheduled visits may occur based on urgent clinical needs. The outpatient clinic staff will be composed of a multidisciplinary team of pediatricians, psychologists, occupational therapists, speech therapists, physiotherapists and nurses. Mothers will be assessed for psychological effects of COVID-19 infection using Edinburgh Postnatal Depression Scale [42] more than once during the first 6 months of their child , as well as breastfeeding and weaning patterns, dietary habits, nutritional status and vaccinal status will be assessed throughout follow-up.

The assessment of the child's neurodevelopment will be carried out up to the 60th month of life. It will include cognitive, motor, socioemotional and language-related aspects and adaptive behavior. The evaluation will be conducted through the Bayley III Child Development Scale version validated for Brazilian infants [43]. From the age of two and a half years onward, aspects related to intellectual performance will also be assessed through the Wechsler Pre-School and Primary Intelligence Scale - 3rd edition, in a six-month interval [44].

CNS imaging assessment will be carried out using a transcranial ultrasound doppler scan

performed between the 15th and 90th day of the child's life. A brain MRI will be performed if altered measures of cephalic perimeter, neurological development delay or abnormal ultrasound doppler scan finding.

In months 12 and 24, the exposed group of children will have blood collections for assessment of their metabolic profile to identify long-term effects on systemic metabolism that are potentially driven by past viral exposure and/or its associated inflammatory response. The examination will consist of assessments of energy metabolism (serum lipids, glucose, insulin), thyroid function (TSH and free T4), bone metabolism (PTH, calcium, phosphate, alkaline phosphatase, 25-hydroxyvitamin D), adrenal tonus (ACTH and basal cortisol), and renal function (blood urea nitrogen, creatinine and urine analysis).

All newborns will undergo extended hearing screening. Evoked otoacoustic emissions and brain shift auditory evoked potential tests will be performed in the first year of life.

Statistical Analysis

All data will be storage in REDCap® (Research Electronic Data Capture) for building and managing online surveys and databases. All variables will be summarized through standard descriptive techniques according to their type and distribution. In the bivariate analysis, differences in categorical variables between exposed and unexposed groups will be verified with the chi-square (or Fisher's exact) test, whereas differences in continuous variables between groups will be assessed with Student’s t-test (or the Mann-Whitney U test).

For dichotomous outcomes, binomial regression models, adjusted for unbalanced relevant background features across the comparison groups, will be used to estimate the relative risks between exposed and nonexposed groups. Partial correlation and/or general linear models will be used to assess associations between continuous outcome variables and covariates, with adjustments for imbalances as appropriate. A p-value of less than 0.05 will be considered significant. The control group will be composed by the same population with the same inclusion criteria – except for the

serology test (IgG,IgM) negative, which will be done at 14-21 days post-partum. The groups won't be matched / paired by age or other variables. However, any distortion between the groups will be adjusted later by statistical means.

Results

This protocol is in the data collection phase.

Study recruitment started in July 2020. As of January 2021 a total of 260 pregnant women infected with SARS-CoV-2 during pregnancy and 180 newborns had been included from hospitals in the Federal District in Brazil. Data analysis is scheduled to start after all data have been collected.

Discussion

The PROUDEST study offers comprehensive insight, from both the obstetric and pediatric perspectives, about the effects of SARS-CoV-2 on the global health of pregnant women and their offspring. Specifically, the study will fill the deep gap in knowledge about the consequences of COVID-19 acquired during early gestation, when the critical stages of embryogenesis take place, as women in all stages of pregnancy will be followed up.

The inflammatory and immunological phenomena induced by the virus in the exposed mother during this early period of life may have a particular impact on the placental and fetal physiology or even be associated with epigenetic signals, which could conceivably determine long-term outcomes in terms of the child's growth, development and metabolism.

A better understanding of these potential long-term consequences requires lengthy, prospective observational studies, such as the PROUDEST study. This study will not only address the clinical outcomes associated with in-pregnancy exposure to COVID-19 but also evaluate a host of soluble and tissue biomarkers, as described in this protocol, aiming at a more comprehensive understanding of the mechanisms underlying the clinical phenomena.

The data will contribute to the overall clinical and basic knowledge of COVID-19, with the ultimate goal of providing grounds to better manage SARS-CoV-2-exposed pregnant women and their children, whether directly or in setting the stage for additional studies. In fact, the PROUDEST study opens up a broad spectrum of possibilities for further research on SARS-CoV-2 effects on maternal, fetal and pediatric health in the context of a multidisciplinary approach. Furthermore, the results might prove relevant from a social perspective, offering data support for the tailored development and implementation of health policies specifically oriented to this particular demographic group.

The study does have some limitations. Its observational nature limits the inference of causal associations to some extent. However, the cohort design is the closest observational equivalent to a clinical trial in terms of analytical power, and the objective of the PROUDEST study does not ethically allow for an interventional experiment because that would imply randomizing pregnant women to be infected by the wild virus. Moreover, the purpose of the study is to characterize the clinical and pathophysiological phenomena associated with COVID-19 in pregnancy and infancy, not to test the efficacy of any intervention. Thus, we deem the cohort design as the best possible design to address our objectives. The lack of random allocation between comparison groups will be partially compensated by statistically adjusting for the observed imbalances. Vaccination for SARS-CoV-2 won’t be an exclusion criteria because There aren’t feasible methods to estimate the proportion of pregnant women that will receive the vaccine, considering its access and availability for now. It can be adjusted and analyzed in small groups of control that have received vaccine or not.

The protracted follow-up in the BORN substudy is expected to result in some dropout. We set a sample size for the study, taking into consideration a 20% rate of loss to follow-up. This might ensure that a sizable number of children reach the final assessment at the age of 5 years, although it cannot avoid “survival bias” in the long-term data. Nevertheless, given that continuity of multidisciplinary assistance will be guaranteed for all children in the BORN branch throughout the

study period, regardless of withdrawal from the analysis or (temporary) loss to follow-up, we expect that children experiencing health problems associated with in-pregnancy COVID-19 exposure will be less likely to drop out than those who are in perfect health. Therefore, we do expect to keep a sufficient number of children in the long run to spot developmental abnormalities (should they exist), despite some amount of dropout.

Some routine pediatric consultation will be emphasized like check the kind of alimentation the child is receiving (human milk, formula milk) and also stimulate the practice of breastfeeding.

Conclusions

The PROUDEST study is a long-term, prospective, cohort study designed to provide a comprehensive view of the effects of in-pregnancy exposure to COVID-19 on women and their offspring from a clinical and pathophysiological standpoint. The results might contribute to improving the management of these exposed mother-child dyads, whether directly or by setting the stage for future specific studies, based on the knowledge of the clinical-pathophysiological phenomena associated with COVID-19 exposure in this particular population.

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCC	blood cell count
CNS	central nervous system
COVID-19	coronavirus disease 2019
CPR	C reactive protein
CSF	cerebrospinal fluid
MRI	magnetic resonance imaging

RT-PCR	reverse transcriptase – polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TSH	thyroid-stimulating hormone

Declarations

Ethics Approval and Consent to Participate

The study was approved by the Research Ethics Committee of the University of Brasilia School of Medicine (http://www.fm.unb.br/cep-fm - CAAE 32359620.0.0000.5558). It was also registered on the Brazilian Register of Clinical Trials (REBEC – https://ensaiosclinicos.gov.br/rg/RBR-65qxs2/). All pregnant women participating in the PROUDEST study are required to sign an informed consent form to join the PREGNANT branch. Likewise, participation of the children in the BORN branch will require signed informed consent from their mothers. Six-month reports on the research status and its partial results will be made available to the institutional Research Ethics Committee and may be publicly consulted upon request.

Consent for Publication

Not applicable

Availability of Data and Materials

At the time of publication of this protocol, the study enrollment and data collection have already started, but we have not completed participant recruitment and the data analysis. Therefore, data sharing is not yet feasible, as no datasets have been generated or analyzed at this stage of the current study. As partial data become available, they will be displayed in REBEC (https://ensaiosclinicos.gov.br/rg/RBR-65qxs2/).

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Geraldo Fernandes, Lizandra Sasaki and Felipe Motta equally contribute to this study and are co-first authors.

Licia Mota, Cleandro Albuquerque and Alberto Carlos Zaconetta equally contribute to this study.

Contributions

Drafting and finalization: GM, LS, FM, ACZ, LMHM

Inputs: CPA, GM, LS, ACZ, MECC

Critical review: AASS, COA, DAAJ, JALJ, RMT, LCGC, CPA

Insightful contributions: all authors

All authors have read and approved the manuscript

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References

1. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. J Infect Public Health. 2020;13:667-73.
2. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433.
3. Whittaker A, Anson M, Harky A. Neurological manifestations of COVID-19: a systematic review and current update. Acta Neurol Scand. 2020;142:14-22.
4. Acharya A, Kevadiya BD, Gendelman HE, Byrareddy SN. SARS-CoV-2 infection leads to neurological dysfunction. J Neuroimmune Pharmacol. 2020;15:167-73.
5. Dashraath P, Wong JLJ, Lim MXK, Lim LM, Li S, Biswas A, et al. Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. Am J Obstet Gynecol. 2020;222:521-31.
6. Li Y, Zhao R, Zheng S, Chen X, Wang J, Sheng X, et al. Lack of vertical transmission of severe acute respiratory syndrome coronavirus 2, China. Emerg Infect Dis. 2020;26:1335-6.
7. Chen D, Yang H, Cao Y, Cheng W, Duan T, Fan C, et al. Expert consensus for managing pregnant women and neonates born to mothers with suspected or confirmed novel coronavirus (COVID-19) infection. Int J Gynaecol Obstet. 2020;149:130-6.
8. Amatya S, Corr TE, Gandhi CK, Glass KM, Kresch MJ, Mujsce DJ, et al. Management of newborns exposed to mothers with confirmed or suspected COVID-19. J Perinatol. 2020;40:987-96.
9. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. Ultrasound Obstet Gynecol. 2020;56:15-27.

10. Xie M, Chen Q. Insight into 2019 novel coronavirus - an updated interim review and lessons from SARS-CoV and MERS-CoV. Int J Infect Dis. 2020;94:119-24.

11. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol. 2001;185:1118-23.

12. Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, et al. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet. 2009;374:451-8.

13. Favre G, Pomar L, Musso D, Baud D. 2019-nCoV epidemic: what about pregnancies? Lancet. 2020;395:e40.

14. Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci. 2011;1221:80-7.

15. Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, Aplin JD, et al. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. FASEB J. 2012;26:4876-85.

16. Romero R, Espinoza J, Gonçalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med. 2006;11:317-26.

17. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol. 1998;179:80-6.

18. Kalagiri RR, Carder T, Choudhury S, Vora N, Ballard AR, Govande V, et al. Inflammation in complicated pregnancy and its outcome. Am J Perinatol. 2016;33:1337-56.

19. Yang X, Yang Y, Yuan Y, Liu L, Meng T. The roles of uterine natural killer (NK) cells and KIR/HLA-C combination in the development of preeclampsia: a systematic review. Biomed Res Int. 2020;2020:4808072.

20. Tomimatsu T, Mimura K, Matsuzaki S, Endo M, Kumasawa K, Kimura T. Preeclampsia: maternal systemic vascular disorder caused by generalized endothelial dysfunction due to

placental antiangiogenic factors. Int J Mol Sci. 2019;20:4246.

21. Gierman LM, Silva GB, Pervaiz Z, Rakner JJ, Mundal SB, Thaning AJ, et al. TLR3 expression by maternal and fetal cells at the maternal-fetal interface in normal and preeclamptic pregnancies. J Leukoc Biol. 2020; doi:10.1002/jlb.3ma0620-728rr.

22. De Rose DU, Piersigilli F, Ronchetti MP, Santisi A, Bersani I, Dotta A, et al. Novel coronavirus disease (COVID-19) in newborns and infants: what we know so far. Ital J Pediatr. 2020;46:56.

23. Zeng L, Xia S, Yuan W, Yan K, Xiao F, Shao J, et al. Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. JAMA Pediatr. 2020;174:722-5.

24. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. JAMA. 2020;323:1846-8.

25. Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, La Rosa M. Severe COVID-19 during pregnancy and possible vertical transmission. Am J Perinatol. 2020;37:861-5.

26. Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental pathology in COVID-19. Am J Clin Pathol. 2020;154:23-32.

27. Baergen RN, Heller DS. Placental pathology in Covid-19 positive mothers: preliminary findings. Pediatr Dev Pathol. 2020;23:177-80.

28. Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E, et al. Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. JAMA. 2020;323:2198-200.

29. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 among children in China. Pediatrics. 2020;145.

30. Yang P, Wang X, Liu P, Wei C, He B, Zheng J, et al. Clinical characteristics and risk assessment of newborns born to mothers with COVID-19. J Clin Virol. 2020;127:104356.

31. Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. Clinical characteristics of 19 neonates born to

mothers with COVID-19. Front Med. 2020;14:193-8.

32. Romagano MP, Guerrero K, Spillane N, Kayaalp E, Smilen SW, Alvarez M, et al. Perinatal outcomes in critically ill pregnant women with coronavirus disease 2019. Am J Obstet Gynecol MFM. 2020;2:100151.

33. Chen Y, Peng H, Wang L, Zhao Y, Zeng L, Gao H, et al. Infants born to mothers with a new coronavirus (COVID-19). Front Pediatr. 2020;8:104.

34. Wang S, Guo L, Chen L, Liu W, Cao Y, Zhang J, et al. A case report of neonatal 2019 coronavirus disease in China. Clin Infect Dis. 2020;71:853-7.

35. Zhu H, Wang L, Fang C, Peng S, Zhang L, Chang G, et al. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatr. 2020;9:51-60.

36. Carvalho WB, Gibelli M, Krebs VLJ, Calil V, Johnston C. Expert recommendations for the care of newborns of mothers with COVID-19. Clinics (Sao Paulo). 2020;75:e1932.

37. Arnaez J, Montes MT, Herranz-Rubia N, Garcia-Alix A. The impact of the current SARS-CoV-2 pandemic on neonatal care. Front Pediatr. 2020;8:247.

38. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal screening for SARS-CoV-2 in women admitted for delivery. N Engl J Med. 2020;382:2163-4.

39. BRASIL - Ministério da Saúde -MS/SVS/DASIS. Sistema de informações sobre nascidos vivos - SINASC. 2020. http://http//tabnet.datasus.gov.br/cgi/tabcgi.exe?sinasc/cnv/nvdf.def. Accessed 25 Oct 2020.

40. Beck, A. T., Steer, R. A., & Brown, G. K. (1996). Manual for the Beck depression inventory-II. San Antonio, TX: Psychological Corporation.

41. Khong, T. Y., Mooney, E. E., Ariel, I., Balmus, N. C. M., Boyd, T. K., Brundler, M. A., Derricott, H., Evans, M. J., Faye-Petersen, O. M., Gillan, J. E., Heazell, A. E. P., Heller, D. S., Jacques, S. M., Keating, S., Kelehan, P., Maes, A., McKay, E. M., Morgan, T. K., Nikkels, P. G. J., ... Gordijn, S. J. (2016). Sampling and definitions of placental lesions Amsterdam

placental workshop group consensus statement. Archives of Pathology and Laboratory Medicine, 140(7), 698-713.

42. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150:782-6.

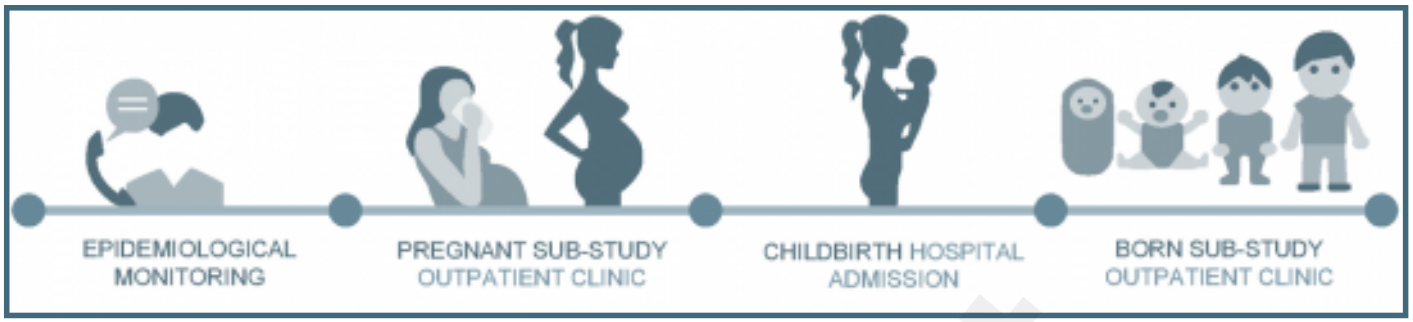
43. Madaschi V, Mecca TP, Macedo EC, Paula CS. Bayley-III scales of infant and toddler development: transcultural adaptation and psychometric properties. Paidéia (Ribeirão Preto). 2016;26:189-97.

44. Karino CA, Laros JA, de Jesus GR. Evidências de validade convergente do SON-R 2½-7[a] com o WPPSI-III e WISC-III. Psicol Reflex Crít. 2011;24:621-9.

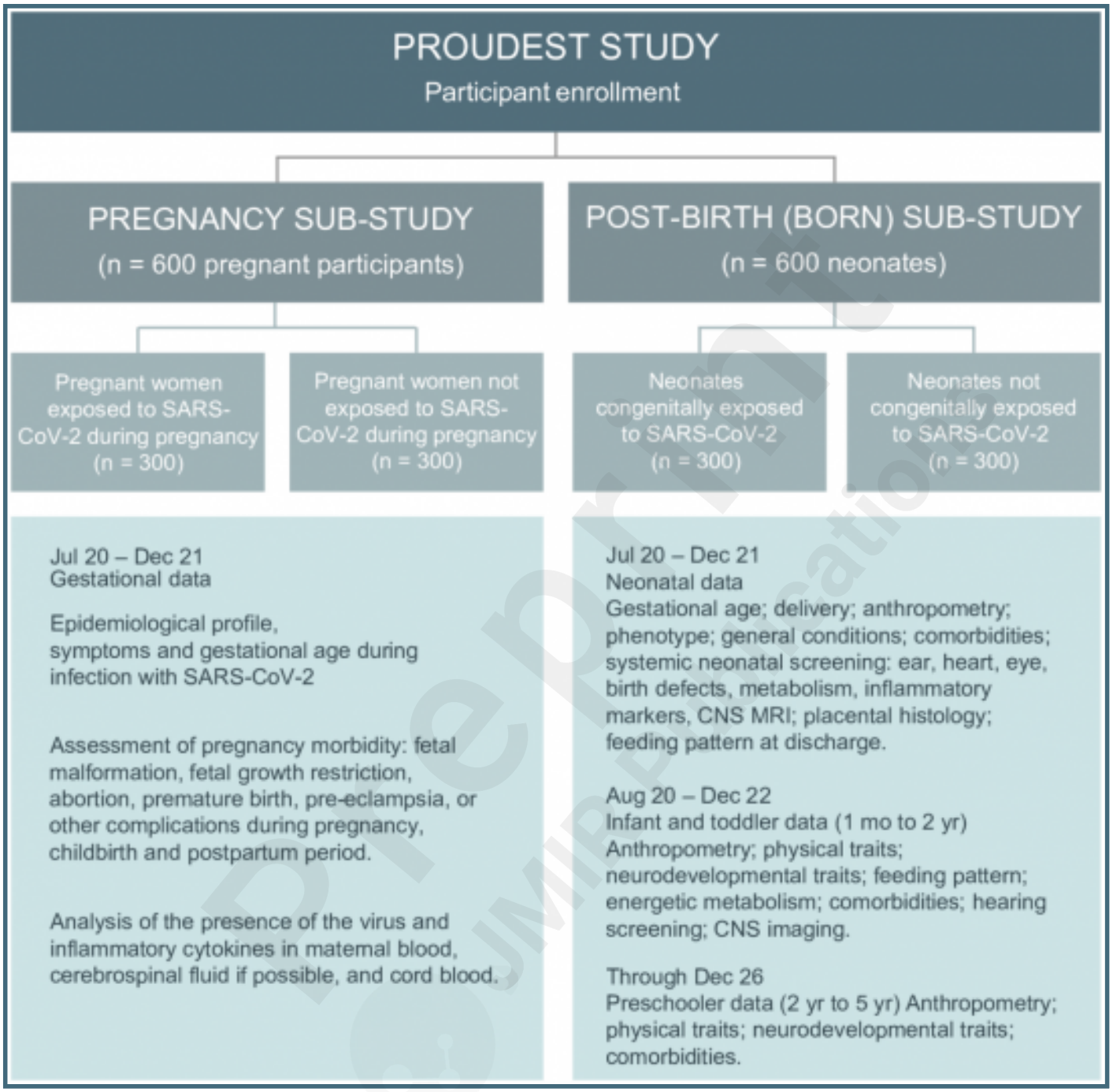
Supplementary Files

Figures

Proudest follow-up flowchart.



Proudest study design.



CONSORT (or other) checklists

Untitled.
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